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STRESS-INDUCED CHANGES IN ERYTHROCYTES. I. EFFECT OF PARACETAMOL AND ITS PRO-DRUG PROPACETAMOL ON GLUTATHIONE CONTENT IN RAT ERYTHROCYTES UNDER COLD/RESTRAINED STRESS

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It is well known that stress lies in the pathogenesis of numerous diseases. Erythrocytes (Er) are very sensitive to oxidative stress expressed with increased hemolysis. This phenomenon is due to reduced antioxidant defense reflecting mainly in diminished intracellular glutathione content. Paracetamol is one of the widely used analgesics-antipyretics. Its pro-drug propacetamol is water-soluble, injectable form of paracetamol recently introduced into the clinical practice for pain relief in the post-operative period. In our study we used the cold/restrained stress (CRS) experimental model to investigate the effects of paracetamol and propacetamol on the glutathione antioxidant system in rat Er. The test drugs were applied in equivalent doses of 250 and 500 mg/kg body mass, respectively, 1 hour before the CRS. After the stress period (4 hours, 4°C) blood was taken and glutathione content in Er-lysates was estimated. The results showed a reduced glutathione content in the CRS group. Pretreatment with paracetamol diminished this parameter by 34 %, while the administration of propacetamol decreased the glutathione concentration in Er more stronger - by 64 %. Our results suggested that CRS had induced dysbalance in Er glutathione antioxidant system which was aggravated by paracetamol and propacetamol.

Key-words: Paracetamol, propacetamol, erythrocytes, glutathione, cold stress

In recent years the role of stress in the pathogenesis of numerous diseases is an area of increasing interest and intensive investigation. Erythrocytes (Er) are very sensitive to oxidative stress and if they cannot defend itself adequately the result is an increased hemolysis. Since red blood cells are exposed to free radicals they are equipped with defense mechanisms against these toxic species. Glutathione system being responsible to maintain adequate levels of reduced glutathione (GSH) is the main antioxidant (AO) defense system in Er. Various stress factors such as temperature, prooxidants, immobilization, etc. may induce dysbalance in this system and this

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reflects in accelerated destruction of these cells.

Paracetamol is one of the widely used analgesics-antipyretics. As it is poorly soluble in water, its usage is limited to oral and rectal routes. In contrast, its pro-drug prapacetamol is a soluble and injectable form which gives it the advantage to be used for pain relief in the post-operative period. There are data available about pro-oxidative properties of paracetamol and it has been proposed to provoke cellular oxidative stress (5,6,10). The effects of paracetamol and moreover propacetamol on the parameters estimating the AO defense in Er are incompletely understood, and the literature data concerning this topic are very scanty. In order to clarify the influence of paracetamol and propacetamol on AO defense of Er we estimated the effects of these drugs on glutathione system in red blood cells under conditions of cold/restrained stress.

MATERIAL AND METHODS

A. Experimental animals

Male Wistar rats weighing 240-270 g were used. The animals were housed at a temperature of 20-40°C, 12-hour dark/light cycle, humidity of 65 % and standard food diet. They were deprived of food 24 hours before the experiment at a free access to water. The test substances were orally (p.o.) applied in a volume of 0,2 ml/100 g one hour before the CRS. Paracetamol (UPSA) was given in a dose of 250 mg/kg b. m., while propacetamol (UPSA) was administered in a dose of 500 mg/kg b. m. corresponding to that of paracetamol of 250 mg/kg b. m.

B. Cold/restrained stress (CRS)

For modeling oxidative stress we used the cold/restrained stress model. The animals were placed in individual restrained plastic boxes sized of 20 x 10 x 10 cm for 4 hours. The experimental procedure was performed following the requirements for humanity of the animal experiments. After the stress period the animals were sacrificed by rapid decapitation.

C. Controls

Two animal groups were used as controls: control animals given only a vehicle instead of the test-drugs in the same test schedule and conditions (Cs), and control ones not treated with the test-drugs and not subjected to CRS (Co).

D. Biochemical analysis

Reduced (GSH) and oxidized (GSSG) glutathione content was determined in Er- hemolysates using heparinized blood taken immediately before the decapitation.

1. Preparation of the hemolysates

Plasma was separated by centrifugation at 2000 rpm for 10 min. Physiologic saline solution (pH=7,4) was used for washing the Er triply. To the pellet of washed Er, equal volume of cold distilled water was added, vortexed and frozen at -20°C for 30 min. Immediately after thawing the hemolysates were used for GSH and GSSG determination.

2. Determination of reduced (GSH) and oxidized (GSSG) glutathione

GSH and GSSG content were assayed according to the method of Hissin and Hilf (7) using o-phthaldialdehyde as fluorescent agent. Standard solutions of GSH and GSSG were applied to calculate the glutathione content in Er-lysates.

E. Statistical analysis

Data were analyzed statistically by Student's *t*-test using a Prism IBM-compatible program. A value of $p < 0,05$ was considered statistically significant.

RESULTS AND DISCUSSION

It has been shown that CRS induced changes in glutathione AO system in Er expressed by reduction of both GSH and GSSG compared to the control Co (Table 1). The percentage of this reduction is 33 % for GSH and 19 % for GSSG. The total glutathione concentration (GSH+GSSG) was diminished by 26 %. Our results indicated an additional reduction in glutathione levels when the animals were pretreated with paracetamol and propacetamol (Table 1). The total gtutathione content was reduced in the paracetamol-pretreated group by 34 % but the effect was more pronounced on GSSG content (65 %). Propacetamol more strongly affected glutathione concentrations in Er than paracetamol. The changes of the analysed parameters were significantly decreased in comparison to the control Cs (Table 1). GSH was reduced by 34 %, GSSG - by 88 %, and the total glutathione content - by 64 %.

There are evidences that AO defense of various cells was reduced by cold stress (8,9). The glutathione system plays central role in AO defense of Er. In the present study it has been shown that CRS reduces both GSH and GSSG suggesting oxidative stress development.

Paracetamol exerts prooxidant properties due to its reactive electrophilic metabolite N-acetyl-p-benzoquinone imine (NAPQI) which is eliminated as glutathione conjugate. A reduction of intracellular glutathione content and altered activities of glutathione reductase and glutathione-S-transferase, the enzymes responsible to maintain adequate levels of intracellular GSH and GSSG is considered a common effect of paracetamol on liver cells (4-6,10). Our results indicate similar effect of paracetamol in Er resulting in diminished glutathione concentration. There are data about a reduced activity of glutathione reductase and increased Er hemolysis as effects of paracetamol action (1-3).

Propacetamol hydrochloride undergoes a rapid and complete hydrolysis due to the non-specific plasma esterases liberating paracetamol and diethylglycyl. Therefore, propacetamol has to be considered devoided of intrinsic toxicity and almost identical to the toxicity of paracetamol. For this reason it is not surprisingly that the effect of propacetamol on Er glutathione content is similar to that of paracetamol. At this stage of our study is very difficult to completely explain the mechanisms by which propacetamol induces a more pronounced reduction in glutathione AO system in Er.

The present paper demonstrates that CRS provokes dysbalance in Er glutathione system and this effect is more manifested when paracetamol (250 mg/kg) and propacetamol (500 mg/kg) are additionally applied.

Table 1

Effect of CRS, paracetamol, and propacetamol on GSH and GSSG content in Er-lysates in rats

Animal groups	GSH in Er-lysates	GSSG in Er-lysates	GSH+GSSG in Er-lysates
Control (Co)*(n=6)	161,70 ± 12,41	171,10 ± 28,18	332,80 ± 20,20
Control (Cs)# (n=6)	108,40 ± 28,40	138,9 ± 37,72	247,30 ± 33,06
Pretreated with paracetamol (n=6)	114,70 ± 25,29	47,65 ± 13,87*	162,35 ± 19,60*
Pretreated with propacetamol (n=6)	71,47 ± 22,25*	16,64 ± 4,70**	88,11 ± 13,40*#

Paracetamol and propacetamol are used in doses of 250 mg/kg b. m. and 500 mg/kg b. m., respectively. GSH and GSSG contents are expressed as g/ml hemolysate. The mean values as well as the range are shown. *$p < 0,05$; **$p < 0,01$ compared to Co; #$p < 0,05$ compared to Cs.

REFERENCES

1. Altorjay, I, L. Dalmi, B. Sari, S. Imre, G. Balla. *Acta Physiol. Hung.*, **80**, 1992, 375-380.- 2. Altorjay, I., B. Sari, S. Imre, G. Balla, L. Dalmi. *Orv. Hetil.*, **134**, 1993, 2259-2262.- 3. Bernal, M., B. Siems, et al. *Ann. Esp. Pediatr.*, **39**, 1993, 501-505.- 4. Bessems, J., T. Koppele, et al. *Xenobiotica*, **26**, 1996, 647-666.- 5. Engelhardt, G., D. Homma. *Arzneimittelforschung*, **45**, 1996, 513-518.- 6. Estevez, F, T. de Angelo, E. Vessel. *Acta Physiol. Pharmacol. Ther.*, **44**, 1994, 48-54.- 7. Hissin, P., A. Hilf. *Anal. Biochem.*, **74**, 1976, 214-222.- 8. Siems, W., R. Brenke. *Arctic Med. Res.*, **51**, 1992, 3-9.- 9. Siems, W., et al. *Free Radic. Biol. Med.*, **16**, 1994, 299-305.- 10. Wang, H., R. Peng. *Chung Kuo Yao Li Hsuch Pao*, 1993, Suppl.1, 41-44.

Стрес-индуцирани промени в еритроцити. I. Ефект на парацетамол и пропацетамол върху концентрациите на глутатиона в еритроцити на плъхове при имобилизационно-студов стрес

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Резюме: Известно е, че стресът лежи в основата на патогенезата на редица заболявания. Еритроцитите са особено чувствителни към оксидативен стрес, водещ до повишена хемолиза. Този феномен се дължи на намалената антиоксидантна защита в резултат на понижаване нивото на вътреклетъчния глутатион. Парацетамолът е един от широко използваните аналгетици-антипиретици. Неговото про-лекарство, пропацетамол, е водно-разтворима инжекционна форма на парацетамола и неотдавна се въведе в клиничната практика за лечение на следоперативната болка. В настоящото изследване бе използван експерименталният модел на имобилизационно-студовия стрес (ИСС) за установяване на ефектите на парацетамола и пропацетамола върху глутатионовата антиоксидантна система в еритроцитите на плъхове. Изследваните препарати бяха прилагани орално в еквивалентни дози (съответно по 250 и 500 mg/kg т. м.) 1 час преди предизвикването на стреса. След ИСС (4 часа при 4^0C) се вземаше кръв и се изследваше концентрацията на глутатиона в еритроцитните хемолизати. Получените резултати показаха намаление на еритроцитния глутатион при животните, подложени на ИСС. Предварителното приложение на парацетамол понижи този показател с 34 %, докато назначаването на пропацетамола намали по-значително концентрацията на глутатиона в еритроцитите (с 64 %). Тези резултатити показаха, че ИСС предизвиква изразен дисбаланс в глутатионовата еритроцитна антиоксидантна система, който се задълбочава от парацетамола и пропацетамола.